# Supplemental Information

Here we provide an assessment of the error associated with the rate constant and reorganization energy determination; full-widths at half height and the peak shifts for the mutant voltammogram as a function of the scan speed, along with a table of FWHM data for different monolayer films; and the dependence of the current on scan speed.

**1. χ square analysis:**

In figure 3 of the paper, we fit the experimentally observed peak shifts to the Marcus model predictions to obtain the standard electron transfer rate constant and reorganization energy. The reliability of the fit and parameter values were assessed through a goodness of fit, χ-square, error analysis. The chi-square is defined as[i]

$$\chi^2 = \frac{1}{2n-2}\sum_{i}^{2n}\left(\frac{\left|E^{i}_{Pthe} - E^{i}_{P\exp}\right|}{\sigma}\right)^2$$

in which n is the total cyclic voltammograms used in the analysis, *i* represents the $i^{th}$ scan with a specific scan rate, $E^{i}_{Pthe}$ and $E^{i}_{P\exp}$ represent a theoretical peak position (oxidation or reduction) and experimental peak position (oxidation or reduction) for the $i^{th}$ scan rate. $\sigma$ is the error of peak position determination during analysis of the raw data. The peak positions were determined for each voltammogram by subtracting a baseline, identifying the peak position, and then correcting for iR drop. The estimated error in this determination is 2 to 4 mV.

The parameters, reorganization energy λ and standard rate constant $k^0$, are determined by minimizing the $\chi^2$. Figure S1 plots the $\chi^2$ value as a function of the standard rate constant for different values of the reorganization energy. It is evident

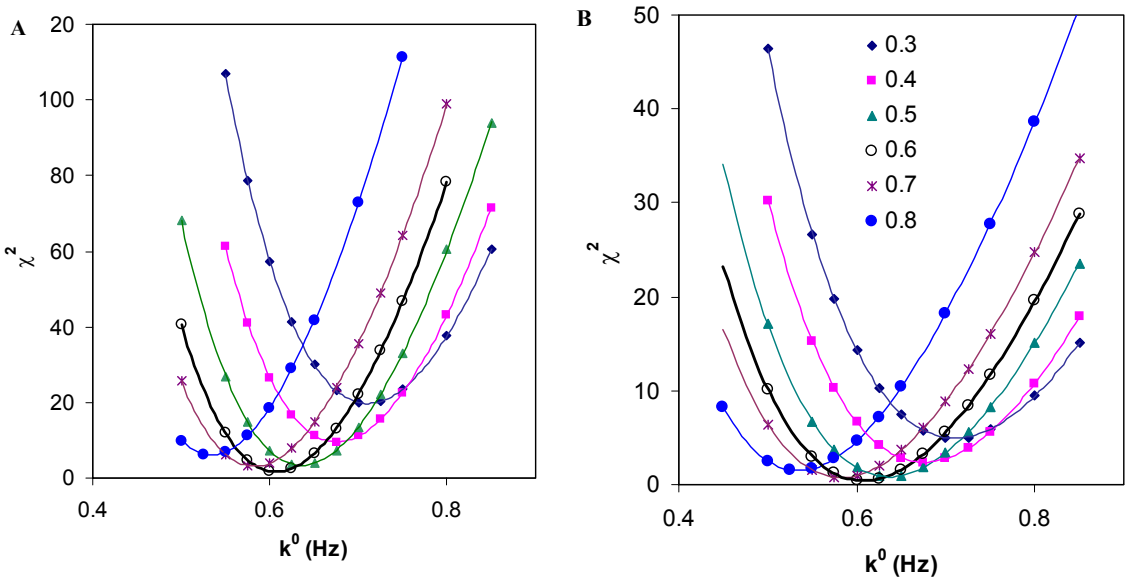


**Fig. S1.** $\chi^2$ analysis of the reorganization energy λ and standard electron transfer rate constant $k^0$. Panel A shows the case for σ= 2 mV, and panel B shows the case for σ=4 mV.

from these graphs that the best fit value of $k^0$ is relatively insensitive (varies by only about 15% with reorganization energy changes of 0.2 eV) to the choice of reorganization energy. The optimized electron transfer rate constant and $\chi^2$ value, at each of the reorganization energies, is reported in Table S1. It is evident from these results that the best fit reorganization energy lies near 0.6 eV. By adjusting both parameters in the fit we find a reorganization energy of 0.58 eV and a standard rate constant of 0.62 $s^{-1}$.

**Table S1: The standard rate constant $k^0$, reorganization energy λ, and $\chi^2$ values at the best fits with the assigned peak errors.**

| $k^0$ | λ | σ = 2 mV | σ = 4 mV |
|---|---|---|---|
| | | $\chi^2_{min}$ | $\chi^2_{min}$ |
| 0.71 | 0.3 | 19.9 | 5.0 |
| 0.68 | 0.4 | 9.7 | 2.3 |
| 0.64 | 0.5 | 3.3 | 0.83 |
| 0.61 | 0.6 | 1.7 | 0.43 |
| 0.58 | 0.7 | 3.0 | 0.79 |
| 0. 53 | 0.8 | 6.0 | 1.54 |

To assess the significance of the $\chi^2$ value, it must be normalized in the proper manner; that is the standard deviation $\sigma$ for the peak assignments must be known. Yet this error will have an element of subjectivity to it because of the data analysis procedure and intrinsic features of the measurement. Assuming that the standard deviation of 2 mV is the correct one, the probability of a $\chi^2$ value greater than 1.7 is only about 2% and values greater than 2.5 is only 0.05 %. Assuming that the standard deviation of 4 mV is the correct one, the probability of a $\chi^2$ value larger than 1 is 46% and a $\chi^2$ value larger than 2 is 0.4%. Hence, a realistic error in the reorganization energy assignment is about 0.1 eV, although it could be as high as 0.2 eV in some cases.

**2. FWHM analysis**

Figure S2 shows the analogue of Figure 3 in the paper for the mutant system. The similarity between the data sets indicates that the pyridyl ligation is similar in both

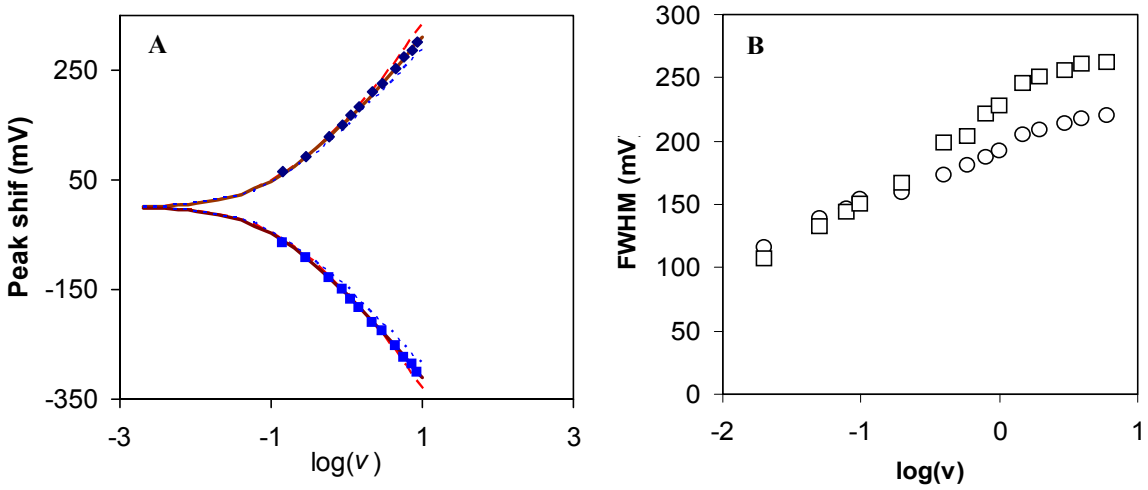


**Figure S2**. Panel A shows the experimental peak shift for mutant K13A rat cytochrome c plotted vs. log($v$) is the voltage scan rate. The three curves are calculated from the Marcus model at reorganization energies: a) 0.3 eV red dashed curve; b) 0.6 eV solid curve, and c) 0.9 eV dotted curve. The best fit is at $k_{et}^0$=0.60 s-1 and reorganization energy 0.65 eV. Panel B shows the increase in the full-width at half maximum for the voltammogram as a function of the scan rate (squares are reduction wave and circles are the oxidation wave).

cases. Table S2 shows the FWHM of the oxidation and reduction waves for the voltammograms obtained on different thickness films at the lowest scan rate studied. A fully reversible process has a FWHM of 90.6 mV and a peak separation of zero.

**Table S2:** the FWHM* (mV) of the oxidation and reduction waves for the voltammograms obtained on different thickness films at low scan rates. The redox reactions are quasireversible.

| Samples | Scan rate (V/sec) | Native rat cyt. C FWHM (mV) | | peak separations | Mutant K13A FWHM (mV) | | peak separations |
|---|---|---|---|---|---|---|---|
| | | Oxidation | Reduction | $E_{pO}$-$E_{pR}$ (mV) | Oxidation | Reduction | $E_{pO}$-$E_{pR}$ (mV) |
| C11Py/C10 | 4 | 104 | 98.6 | 8±2 | 105 | 100 | 9±2 |
| C12Py/C11 | 2 | 106 | 97.6 | 5±2 | 101 | 97 | 6±2 |
| C16Py/C15 | 1 | 108 | 101 | 25±3 | 107 | 102 | 20±3 |

* The deviation for FWHM is about ±3 mV.

**3. Current versus voltage dependence**

The relationship of scan rate (v) vs. peak current (Ip) should be linear for immobilized protein and vary as the square root of peak current for freely diffusing protein. Figure S3 shows this dependence for the mutant K13A on C20Py/C19 SAMs. Panel A shows the linear relationship of scan rate with the peak current (the $R^2$ is 0.992, 0.995 for anodic and cathodic peak currents, respectively). Panel B shows the same data versus the square root of scan rate with the peak currents, ( $R^2$ is 0.962, 0.971 for anodic and cathodic peak currents, respectively). Similar results were obtained for the native rat cytochrome c on the pyridine systems.

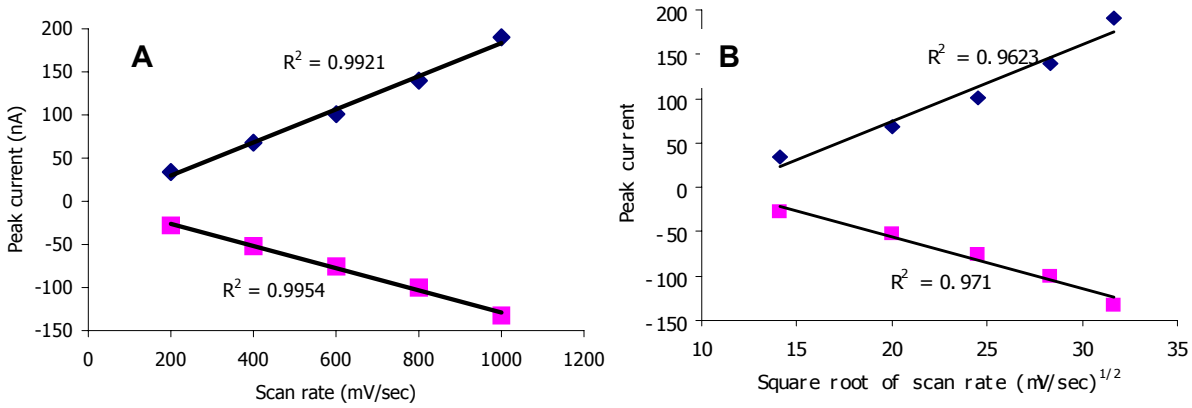


**Figure S3** – The figure compares a plot of peak current versus scan rate to that of peak current versus the square root of scan rate. See text for details.

[i] Taylor, JR An Introduction to Error Analysis, Chapter 12, p261, 2nd edition, University Science Books, **1997**